relevant questions concerning the conduction and selectivity mechanism in the former. So far various efforts have been made towards characterizing these processes, yet there are still aspects that remain to be clarified. Previous metadynamics analysis, as well as other independent studies, of the equilibrium conduction mechanism in NavAb point to a two-ion mechanism, decoupled from other incoming ions and water molecules. From the 0 mV potential of mean force (pmf) relative to the two-ion conduction through the channel SF, we analytically evaluate the pmf's dependence on applied voltage potential (V). When small V is applied biased and unbiased pmfs are overall similar. Contrastingly, under higher V conditions, an excess free energy arising from the applied external potential causes an asymmetry between hyper ($V<0$) and depolarized ($V>0$) conduction free energy surfaces and hence alters conduction mechanism. These results agree with MD studies explicitly considering the influence of transmembrane potentials. Same analysis has been applied to a similar system containing two K^+ inside the NavAb SF. We next aimed at further glancing at the channel conduction and selectivity, by characterizing the kinetics of the process by means of transition state theory. Surprisingly however, under higher voltages, the kinetic model highly overestimate the expected conductance of the channel. We are now faced with the question as to whether the TST model is inaccurate (though it has been previously shown to work), or whether deeper issues, such as mistaking the heights of barriers in the relatively confined SF environment are also playing a role.

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Negative Countercharges and S4 Interaction in Domain IV of Nav1.4

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The voltage sensor module comprises the positively charged S4 residues and negative countercharges in S1 to S3 segments. In the present study the putative interaction of these charges in domain IV during fast inactivation of the human voltage gated sodium channel of skeletal muscle was investigated. The effects of charge reversing mutations of R1448 and R1457 were compared to those for mutations at N1366, E1373, N1389 and D1420. Mutations slowed the entry of channels into fast inactivation, slowed or accelerated the recovery of channels from fast inactivation, and reduced the gating charge. Charge swapping mutations had a more pronounced effect to rescue the wild type phenotype on recovery from fast inactivation than for entry into fast inactivation. These results suggest specific residue interactions that promote S4 translocation during the deactivating transition leading to recovery from the fast inactivated state.

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Modulation of Inactivation Kinetics of the Bacterial Sodium Channel Nachbac Suggests a Complex Mode of Inhibition by Isoflurane

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The bacterial sodium channel NaChBac is a prokaryotic ancestor of eukaryotic voltage-gated sodium channels (Nav), which are critical for action potential generation and propagation in nervous and cardiac tissue. Like all mammalian Nav isoforms examined thus far, NaChBac is inhibited by clinically relevant concentrations of the inhaled volatile anesthetic isoflurane, and inhibition is accompanied by enhancement of slow or C-type inactivation (Ouyang et al., 2007). However, a detailed mechanistic explanation of the interplay between C-type inactivation and isoflurane inhibition is lacking. To shed light on the relationship between inactivation and inhibition, we introduced point mutations known to alter inactivation in NaChBac (G219A, G219P, G229A, and S195E), expressed the channels in HEK293FT cells, and applied isoflurane during whole-cell patch clamp recording. Preliminary data support the idea that isoflurane acts by multiple mechanisms invovling multiple sites on NaChBac. Channel mutations that enhance inactivation show greater current reduction by isoflurane, suggesting that isoflurane binds more favorably to inactivated channels. However, isoflurane exhibits both tonic and use-dependent block of the essentially non-inactivating NaChBac G219P mutant, indicating that binding also occurs in the closed/resting and open/conducting states. A detailed biophysical and pharmacological profile of these NaChBac inactivation variants together with a recent molecular dynamics simulation study showing isoflurane interacting with NaChBac at three distinct binding sites (Carnavale et al, 2013) supports multiple mechanisms of state-dependent inhibtion. Such functional data help clarify the complex pharmacological effects of volatile anesthetics on Nav channels, and contribute to better understanding of C-type inactivation in these proteins.

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Pharmacological Properties of Cinnamaldehyde on NaChBac

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Recent breakthroughs in the structure determination of the bacterial voltage-gated sodium channel NavAb have revealed the full length Nav channel for the first time at atomic resolution. However, additional structures of Nav channels in different conformational states are still needed for further understanding of the conformational changes that take place in Nav channels during channel gating. Specifically, full-length structures of Nav channels in the resting state, open state, inactivated state, and drug-bound conformations are still missing. In our search for ligands that confine Nav channels in one of these conformational states we have turned our attention to cinnamaldehyde. We tested cinnamaldehyde, a compound that affects different subtypes of eukaryote Nav channels, on the bacterial Nav channel NaChBac expressed in Xenopus oocytes. Our results indicate that cinnamaldehyde has a dual effect on NaChBac: cinnamaldehyde not only decreases the peak current but also accelerates channel inactivation of this bacterial Nav channel in a concentration-dependent manner. Our recordings indicate that cinnamaldehyde stabilizes NaChBac in an inactivated state. This opens up perspectives to employ cinnamaldehyde as a molecular tool to aid crystallization of bacterial Nav channels in an inactivated conformation. We expect that such a structure could give important insight into the conformational changes that contribute to channel inactivation.

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Automated Patch Clamping of 384 Cells at Once for Massively Parallel Ion Channel Screening

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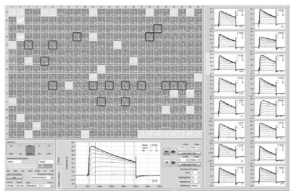
Automated patch clamping is well established within academic research and drug discovery efforts. Still, there is a longstanding desire to have gold standard electrophysiology compatible with primary ion channel drug screening requirements. We here present a chip-based approach for massively parallel patch clamp recordings. Using microstructured glass bottom microtitre plates, recordings from 384 cells are performed in an automated fashion. The recording system contains 384 patch clamp amplifiers and is integrated into a liquid handling robot with 384 channel pipettor, so all experiments are done completely in parallel. Success rates achieved are routinely over 85%. A full run of 384 cells for dose response analysis takes less than 15 minutes, delivering several thousand data points per hour.



The figure below shows a screenshot of an experiment on 384 CHO cells expressing Kv1.3 channels. (Raw current responses to a voltage step protocol). In this presentation, recordings from various ligand- and voltage-gated ion channels on the 384 channel platform will be shown.

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Sodium Channel Peptide Neurotoxin Studies Using a High Throughput Electrophysiology Platform and Very Long Assay Windows

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Neurotoxins identified in various animal venoms have long been used for studying ion channel structure and function, due to the high binding affinity of toxins to ion channels. Over the years, peptide neurotoxins have also attracted great interest as potential strategies for treatment of ion channel-related diseases. Despite the growing need of developing peptide toxins as drug candidates, the high-throughput analysis of peptide toxin can be challenging, as the toxins are typically large molecules with relatively slower binding-rate to the channel targets (compared to small molecule compounds). In addition, the binding affinities of these toxins to the channel are often state-dependent. Taken together, to successfully assay these toxins an assay platform needs to provide: 1) A long and stable assay window to capture the full association kinetics of the toxins, 2) Sophisticated voltage protocols to drive channels into desired conformational states, and 3) Adequate throughput and low running cost requirements of a drug screening environment. In this